ACS APPLIED MATERIALS & INTERFACES

www.acsami.org

Research Article

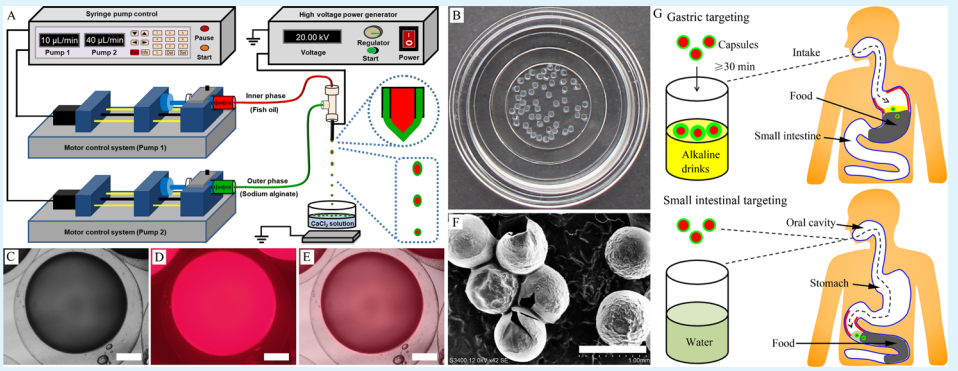
Electrosprayed Soft Capsules of Millimeter Size for Specifically Delivering Fish Oil/Nutrients to the Stomach and Intestines

Panpan Wang,[▽] Min Li,[▽] Daixu Wei, Mengzhen Ding, Lina Tao, Xunwei Liu, Fengping Zhang, Ningping Tao, Xichang Wang, Mingyuan Gao, and Jian Zhong*

Cite This: https://dx.doi.org/10.1021/acsami.9b23623

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information



ABSTRACT: Contrasting to the traditional centimeter-sized soft capsules that are difficult to swallow or micro/nanometer-sized soft capsules that suffer from limited loading capacity for fish oil/nutrients and lowered stability, the millimeter-sized soft capsules with good enough stability could be a potential solution in solving these problems. Herein, we report millimeter-sized soft core−shell capsules of 0.42−1.85 mm with an inner diameter of 0.36−1.75 mm, for fish oil/nutrients, obtained through an electrospray approach upon optimization of different fabrication parameters such as applied voltage, sodium alginate concentration, shell/core feeding rate ratio, times of feeding rate, and types of coaxial needles. Further in vitro and in vivo studies reveal that the resulting soft capsules were apparently weakened and became mechanically destructive in the simulated small intestine solution and were totally destroyed in the simulated small intestine solution if they were first treated in the simulated stomach solution but not in the simulated stomach solution, which makes the millimeter-sized capsules useful as containers for specific delivery of fish oils and lipophilic nutrients to the stomach and intestines with excellent in vivo bioavailability (>90%). The whole fabrication approach is very facile with no complicated polymer modification and formulations involved, which endows the resulting soft capsules with broad application prospect in food and drug industries.

KEYWORDS: *alginate, β-carotene, electrospray, fish oil, soft capsules, targeting*

Downloaded via UPPSALA UNIV on January 26, 2020 at 08:55:16 (UTC).
See https://pubs.acs.org/sharingguidelines for options on how to legitimately share published articles.

1. INTRODUCTION

Soft capsules have been applied to encapsulate active substances in pharmaceutical and food industries due to their huge advantages:[1−3] (i) masking of odor or unhappy taste; (ii) protection of highly reactive ingredients from oxygen, heat, or high/low pH; (iii) controllable release of ingredients; and (iv) conversion of liquid ingredients to solid forms for easy processing and storage. In the academic and industry fields of pharmaceutics and foods, traditional soft capsules of centimeter size and micro/nanocapsules are the mainstream objectives. Traditional soft capsules are difficult to swallow, particularly for infants, children, elderly, and some patients. Micro/nanocapsules are limited by their loading capacities and low stability apart from high energy consumption in fabrication. Therefore, it is meaningful to develop soft capsules of millimeter size to eliminate the difficulty in swallowing compared with traditional soft capsules of centimeter size and increase the loading capacities and stability of fish oil, nutrients, and drugs compared with micro/nanocapsules.

In fact, the millimeter-sized soft capsules are being explored over the past two decades. Nagai et al.[4] developed poly(dimethylsiloxane)-encapsulated poly(amic acid) capsules with a diameter of 0.5−5 mm by injecting poly(dimethylsiloxane) into poly(amic acid) spheres and curing the resulting capsules at high temperature. Bourlinos and Petridis[5] developed millimeter-sized polymeric capsules by dropwise adding an

Received: December 31, 2019
Accepted: January 14, 2020
Published: January 15, 2020

© XXXX American Chemical Society

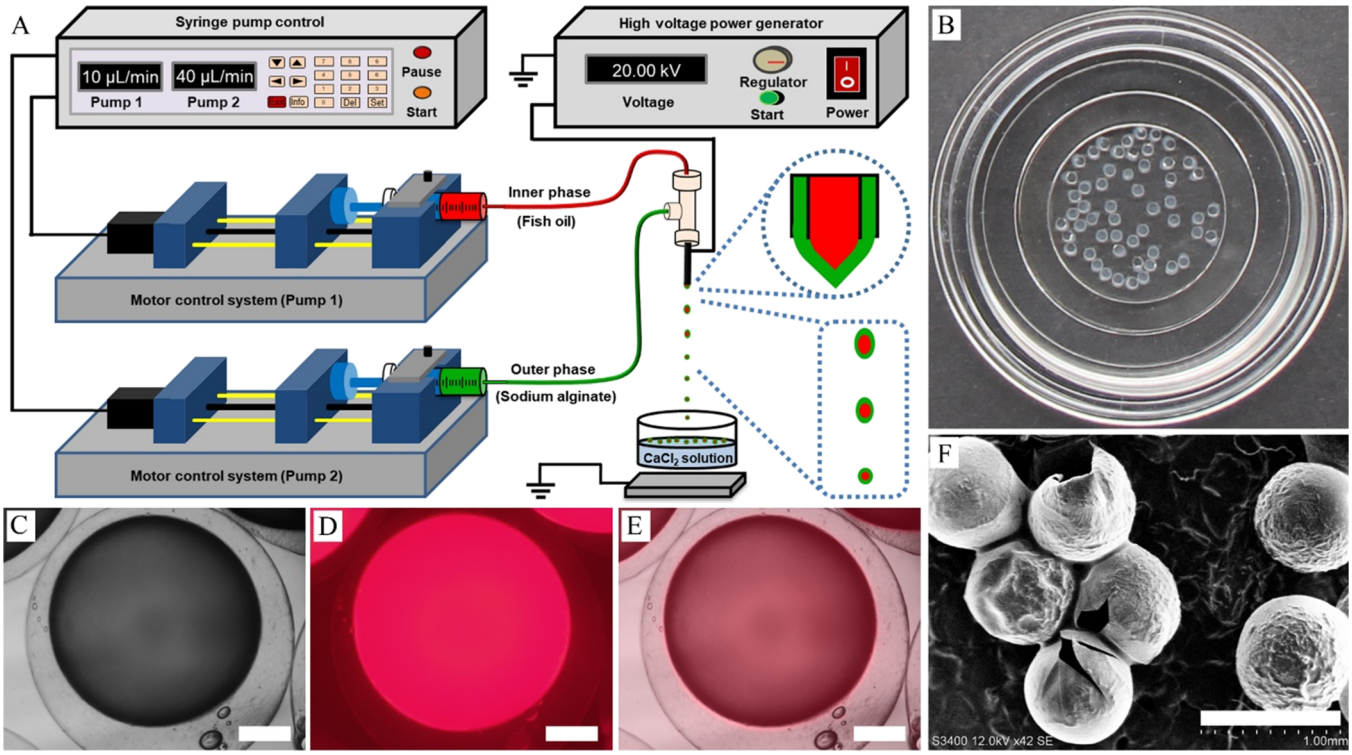


Figure 1. Preparation and confirmation of electrosprayed fish oil-loaded core−shell millimeter-sized soft capsules. (A) Schematic of the coaxial electrospraying instrument for the preparation of core−shell millimeter-sized soft capsules. (B) Typical core−shell millimeter-sized soft capsules in a 35 mm Petri dish. (C−E) phase contrast, fluorescence (fish oils were mixed with Nile Red), and merged images of typical core−shell millimeter-sized soft capsules. Scale bars indicate 0.2 mm. (F) Scanning electron microscopy image of destroyed and undestroyed soft capsules. Scale bar indicates 1.0 mm.

aqueous carboxymethyl cellulose solution into an *n*-butanol solution with copper or iron salt. Recently, millimeter-sized capsules were prepared using liquid marbles by dropwise adding a trimethylolpropane trimethacrylate monomer droplet on superamphiphobic powders, rolling it to form liquid marbles, injecting water droplet into the liquid marbles, and irradiating them by visible light.[6] However, these methods are not suitable for encapsulating oil into millimeter-sized soft capsules.

Over the past decades, electrospinning and electrospraying have drawn increasing attentions. Electrospinning is a simple technique for fabricating nanofibers. In a typical electrospinning process, a stable polymer liquid jet is ejected from a Taylor cone formed at a syringe needle under a high voltage, dried through the air to form nanofibers, and collected on an electrically grounded target.[7] Electrospraying as a variant of electrospinning technique has been developed and used for preparing micro/nanometer micro/nanospheres.[8] Electrospraying generally uses lower polymer concentrations than electrospinning. Electrospinning techniques are widely applied in tissue engineering,[9−11] fast detection,[12−14] air filtration,[15−17] actuators,[18,19] and drug delivery,[20−22] while electrospraying techniques are widely applied in tissue engineering[23,24] and drug delivery.[25,26] To further widen the application of electrospraying, two different strategies have been developed. One is to prepare millimeter-sized spheres by the two-step fabrication method for encapsulation of micrometer-sized samples such as probiotics,[27] while the other is to fabricate microcapsules[28] and nanocapsules[29] by coaxially electrospraying the shell and core substances. However, to our knowledge, this technique has not been used for fabricating core−shell structured soft capsules of millimeter sizes.

2. RESULTS AND DISCUSSION

In this work, we used fish oil as the core for the loading of lipid soluble nutrients and alginate cross-linked by calcium ions as a shell.[30] Because fish oils are rich in omega-3 unsaturated fatty acids, they are important nutrient substances for human beings.[31] However, the low chemical stability of the double bond, fishy taste, and poor water solubility[32] largely restrain the wide applications of fish oils in food and drug industries. By optimizing the fabrication parameters, millimeter-sized soft capsules were obtained with a custom-designed coaxial electrospraying instrument (Figure 1A). The following parameters were optimized: for example, the concentration of sodium alginate solution was of 2%, the applied voltage was of 20 kV, the distance between the coaxial needle and the liquid surface of the collecting solution is of 9 cm, the inner and outer needle of the coaxial needles were 23# and 17#, respectively, and the feeding rates for the inner and the outer phase were 10 and 40 μL/min, respectively.

As shown in Figure 1B, the soft capsules obtained with above fabrication parameters are spherical in shape and very uniform in size. We could not smell the fishy taste, which suggested that the fishy taste of fish oil was masked by the capsule shell. Further fluorescence microscopy experiments (Figure 1C−E) showed that the capsules were completely full of fish oil as the core. According to the phase contrast studies (Figure 1C), the capsule and core diameters were 927 ± 32 (n = 20) and 751 ± 15 μm (n = 20), respectively. After 1 h of drying at room temperature, the soft capsules shrunk and

https://dx.doi.org/10.1021/acsami.9b23623
ACS Appl. Mater. Interfaces XXXX, XXX, XXX−XXX

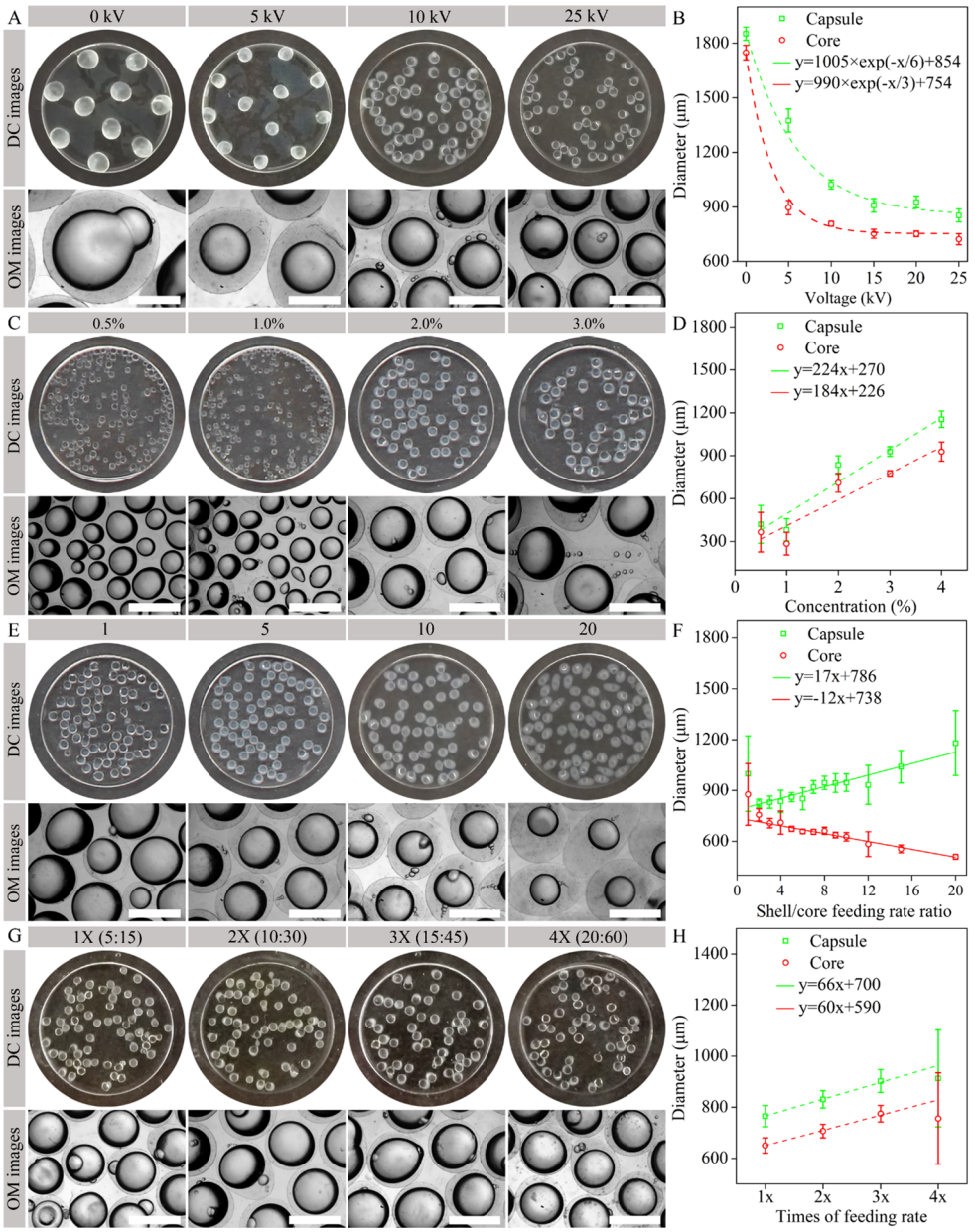


Figure 2. Effect of preparation parameters on fish oil-loaded core−shell millimeter-sized soft capsules. (A) Digital camera and optical microscopy images of typical soft capsules prepared under different applied voltages. (B) Soft capsule and fish oil core diameters as a function of applied voltages. (C) Digital camera and optical microscopy images of typical soft capsules prepared with different sodium alginate concentrations. (D) Soft capsule and fish oil core diameters as a function of sodium alginate concentrations. (E) Digital camera and optical microscopy images of typical soft capsules prepared with a different shell/core feeding rate ratio (the core feeding rate was 10 μL/min). (F) Soft capsule and fish oil core diameters as a function of the shell/core feeding rate ratio (the core feeding rate was 10 μL/min). (G) Digital camera and optical microscopy images of typical soft capsules prepared with different times of feeding rate. The numbers in brackets indicate core feeding rate vs shell feeding rate. (H) Soft capsule and fish oil core diameters as a function of times of feeding rate. All the digital camera images only showed the central part of the 35 mm Petri dish, and the light circles in these images had a diameter of 15 mm. All the scale bars in the optical microscopy images indicate 1.0 mm. DC, digital camera; OM, optical microscopy.

became brittle. Further SEM studies (Figure 1F) revealed that the capsules in a dry state is of 825 ± 37 μm (n = 20). The broken capsules shown in the SEM image suggests that the soft capsules become unstable in a dry state. In addition, environmental calcium could maintain the alginate cross-linking with calcium.[33] Therefore, in below experiments, the soft capsules were stored in the collecting solution (25 mM $CaCl_2$ solution).

To investigate the effects of fabrication parameters on morphology of the electrosprayed fish oil-loaded soft capsules, a series of preparations with varied fabrication parameters were carried out. The obtained soft capsules were spherical or fusiform, whereas the cores were mainly spherical. As shown in Figure 2 and Figures S1 to S6, the diameters of the soft capsule and the oil core are exponentially decreased with the increase of applied voltage (Figure 2A,B and Figure S1) but are linearly increased against the concentration of sodium alginate (Figure 2C,D and Figure S2). In addition, the capsule diameter, core diameter, thickest shell/core thickness ratio, and thinnest shell/core thickness ratio are linearly increased with the

https://dx.doi.org/10.1021/acsami.9b23623
ACS Appl. Mater. Interfaces XXXX, XXX, XXX−XXX

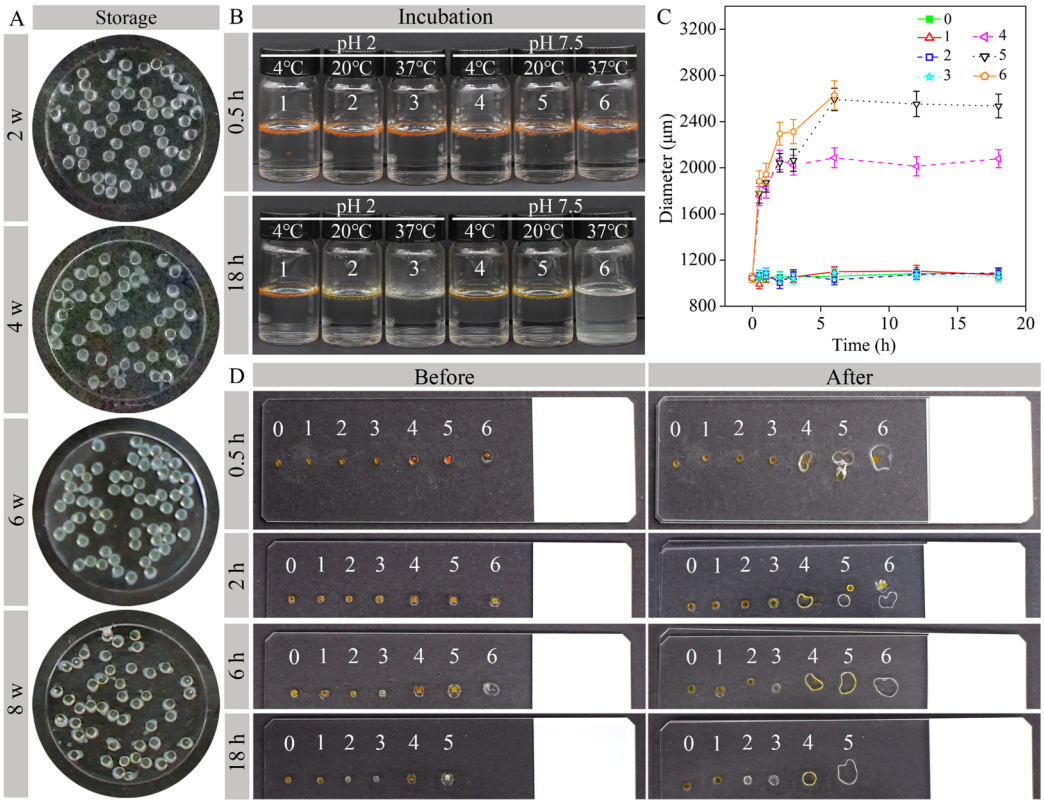


Figure 3. Stability and in vitro digestion of fish oil-loaded core−shell millimeter-sized soft capsules. (A) Soft capsules after different storage times in 25 mM $CaCl_2$ solution. All the digital camera images only showed the central part of the 35 mm Petri dish, and the light circles in these images had a diameter of 15 mm. (B) Fish oil/β-carotene-loaded soft capsules (samples 1−6) after different digestion times in different pH solution at different storage temperatures. (C) Diameters of β-carotene-loaded soft capsules after different digestion times in different pH solution at different storage temperatures in panel (B). Undigested fish oil/β-carotene-loaded soft capsules that were stored in 25 mM $CaCl_2$ solution were used as controls. Samples 1−6 refer as the samples from left to right in panel (B). (D) Bearing tests of fish oil/β-carotene-loaded soft capsules (samples 1−6) after different digestion times in different pH solution at different digestion temperatures. Undigested soft capsules that were stored in 25 mM $CaCl_2$ solution were used as controls (sample 0). One bearing microslide was applied.

increase of the shell/core feeding rate ratio. The resulting fusiform and spherical capsules are shown in Figure 2E,F and Figures S3 and S4, and the capsule and oil core sizes are also linearly increased with the increased times of feeding rate (Figure 2G,H). As expected, the capsule and oil core diameters also present coaxial needle size dependency (Figure S5 and S6). Three combinations of coaxial needles, that is, 23#/17#, 21#/15#, and 23#/15#, were found to give rise to pure calcium alginate spheres when no fish oil was fed. However, only 23#/17# and 21#/15# coaxial needle combinations could give rise to a core−shell type of soft capsules, which is because of the mismatch in the shell/core sectional area ratio and the shell/core feeding rate ratio for the 23#/15# coaxial needle combination. Different distances between the coaxial needle and the liquid surface of the collecting solution had no obvious effects on β-carotene-loaded capsule shapes and structures (Figure S7). The capsule diameters slightly increased with the increase of the collecting distances (Figure S8). All the thicknesses of the calcium alginate shell in this work were uneven. The possible reason might be the collision of the soft capsule on the liquid surface of $CaCl_2$ solution during the electrospraying process. Based on all these effects of different fabrication parameters, millimeter-sized soft capsules with a capsule diameter of 0.42−1.85 mm and an oil diameter of 0.36−1.75 mm were prepared for the following studies.

The stability of fish oil-loaded capsules was analyzed. As shown in Figure 3A and Figures S9 and S10, the capsules are very stable and show near no variation in the capsule size after being kept in 25 mM $CaCl_2$ solution for 8 weeks, suggesting that the storage in the $CaCl_2$ solution is appropriate for maintaining the stability of the oil-loaded capsules. Then, the stability of the fish oil-loaded capsules (preparation parameters: 23#/17# coaxial needle, an applied voltage of 20.0 kV, 2.0% sodium alginate, a core feeding rate of 10 μL/min, a shell feeding rate of 40 μL/min, and a β-carotene/oil mass/volume ratio of 0.83 mg/mL) in simulated stomach (10 mM NaH_2PO_4, pH 2.0, adjusted with phosphoric acid) and small intestine (8.4 mM Na_2HPO_4, 1.6 mM NaH_2PO_4, pH 7.5) solutions was investigated to show their in vivo digestion behaviors. The pH 2.0 and 7.5 were chosen to simulate the gastric environment[34] and intestinal environment.[35] In this study, β-carotene was used as a model for the lipophilic nutraceutical material and was mixed with fish oil prior to the encapsulation. As β-carotene is a color pigment, the color variation can also be used as an indicator for showing the release of the encapsulated oil. As shown in Figure 3B,C and Figure S10, the diameters of the soft capsules (samples 1−3) in the simulated stomach solution (pH 2) had no obvious change compared with the undigestible control (Figure 3C). The soft capsules (samples 4−6) incubated in the simulated small intestine (pH 7.5), however, are remarkably increased in size in comparison with samples 1−3 and the control as well (Figure 3C). The following bearing ability tests (partially to simulate gastrointestinal motility), as shown in Figure 3D, reveal that

https://dx.doi.org/10.1021/acsami.9b23623
ACS Appl. Mater. Interfaces XXXX, XXX, XXX−XXX

the soft capsules kept in the simulated stomach solution (pH 2.5) remain intact and hardly destroyable under a 5 g weight load (one piece of a microslide). In contrast, the soft capsules kept in the simulated small intestine solution (pH 7.5) become largely softened (Figure 3D) and expandable and destroyable under an external 5 g weight load. In particular, the capsules in sample 6 (pH 7.5/37 °C) start to largely expand 6 h after incubation (Figure 3D) and finally become invisible under eyes and digital camera after 12 h (Figure S11). The temporal bearing abilities of these soft capsules are also recorded and shown in Videos S1 to S5.

To simulate the in vivo digestion of soft capsules via gastric and intestinal tract, the in vitro digestion of fish oil/β-carotene-loaded millimeter-sized soft capsules from the simulated stomach solution to the simulated small intestine solution was analyzed (Figure 4). The results showed that, after storage

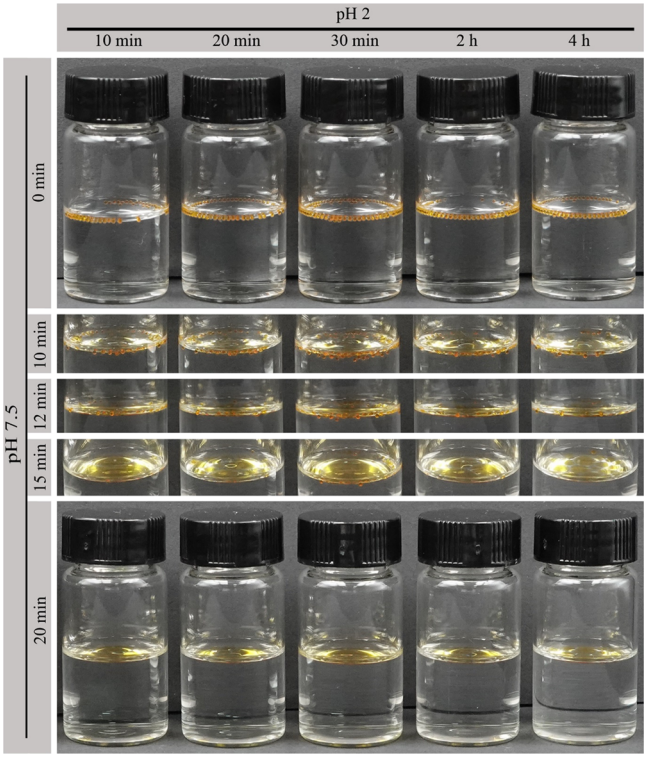


Figure 4. In vitro digestion of fish oil/β-carotene-loaded core−shell millimeter-sized soft capsules in PBS (pH 7.5) at different digestion times (0, 10, 12, 15, and 20 min). Prior to in vitro digestion in PBS (pH 7.5), these soft capsules were stored in PBS (pH 2.0) with different times (10 min, 20 min, 30 min, 2 h, and 4 h).

at PBS (pH 2.0) for 10 min to 4 h, the soft capsules were partially destroyed in 10 min and were destroyed in 20 min. It suggested that fish oil/β-carotene could be delivered to the small intestine via these soft capsules, which did not need any in vitro bearing weight load or potential in vivo intestinal peristalsis.

Rabbit models with empty and full stomachs were constructed (Figure 5A−E), and the in vivo behaviors of these soft capsules in rabbit models were explored (Figure 5F−K). The free flow through distribution of iopromide injection solution (black color in Figure 5A,B) in the stomach area and the presence of barium sulfate in both the stomach and small intestine (white color in Figure 5C) demonstrated that the rabbit model had a good stomach-emptying state. The presence of barium sulfate only in the stomach (white color in Figure 5D,F) indicated that the rabbit model has a good full-stomach state. Therefore, rabbit models with empty and full stomachs were successfully constructed. Untreated, PBS (pH 7.5)-treated, and untreated soft capsules (preparation parameters: 23#/17# coaxial needle, an applied voltage of 20.0 kV, the distance between the coaxial needle and the liquid surface of the collecting solution of 9 cm, 2.0% sodium alginate, a core feeding rate of 10 μL/min, a shell feeding rate of 40 μL/min, and a β-carotene/oil mass/volume ratio of 1.25 mg/mL) were directly into the stomachs of the rabbit model with full stomach, full stomach, and empty stomach, respectively. The soft capsules without pretreatment of PBS were not destroyed in the full stomach even at 48 h after direct perfusion (Figure 5F), whereas the soft capsule with pretreatment of PBS were destroyed in the full stomach at 0.5 h after perfusion (Figure 5G,H). Further, the soft capsules without pretreatment were destroyed in the small intestine at 0.5 h after perfusion (Figure 5I,J).

As shown in Figure 5K, the β-carotene amounts in the rabbit blood increased and then decreased to the original level at 24 h after perfusion. The ideal β-carotene amount (C_1) in rabbit blood if all the β-carotene compounds were released and adsorbed by the rabbit can be calculated by the equation below

$$C_1 = \frac{n \times C_0 \times \frac{4\pi\left(\frac{d}{2}\right)^3}{3}}{V} \quad (1)$$

where n is the number (20, in this work) of the applied soft capsule, C_0 is the β-carotene/oil mass/volume ratio (1.25 mg/mL, in this work), d is the oil core diameter (752 ± 15 μm, in this work) of the applied soft capsule, and V is the blood volume of the rabbit (about 55.65 mL/kg rabbit. So, V was 83.48 mL for 1.5 kg of rabbit in this work). Therefore, the ideal β-carotene amount (C_1) in rabbit blood was 66.68 ng/mL. The practical β-carotene increased amounts (Figure 5K) for the gastric (Figure 5G,H) and intestinal targeting (Figure 5G,H) were 64.50 and 60.89 ng/mL, respectively. Therefore, the practical bioavailability values were 96.73 and 91.31% for gastric and intestinal targeting, respectively. It suggests that lipophilic nutrients in the soft capsules had excellent bioavailability.

All these results suggested that the millimeter-sized soft capsules are small intestine-specific and suitable as containers for delivering fish oil and lipophilic nutrients into the small intestine with excellent bioavailability (Figure 5L), in which the soft capsules become expandable and destroyable upon intestinal peristalsis (Figure 3), or were directly destroyed in the intestinal environment after the gastric environment (Figure 4). The soft capsules can also be potentially used for stomach-specific delivery if they are added into alkaline drinks to swallow the capsules for more than 30 min prior to the delivery with the alkaline drinks after meal (Figure 5L). In the stomach, the expanded capsules will be destroyed by gastric peristalsis to release the payload.

3. CONCLUSIONS

In summary, a facile electrospray-based approach was developed for fabricating millimeter-sized alginate capsules of 0.42−1.85 mm with an inner diameter of 0.36−1.75 mm. The fishy taste of fish oil was masked by the capsule shell. Conversion of liquid fish oil to solid soft capsules was achieved

https://dx.doi.org/10.1021/acsami.9b23623
ACS Appl. Mater. Interfaces XXXX, XXX, XXX−XXX

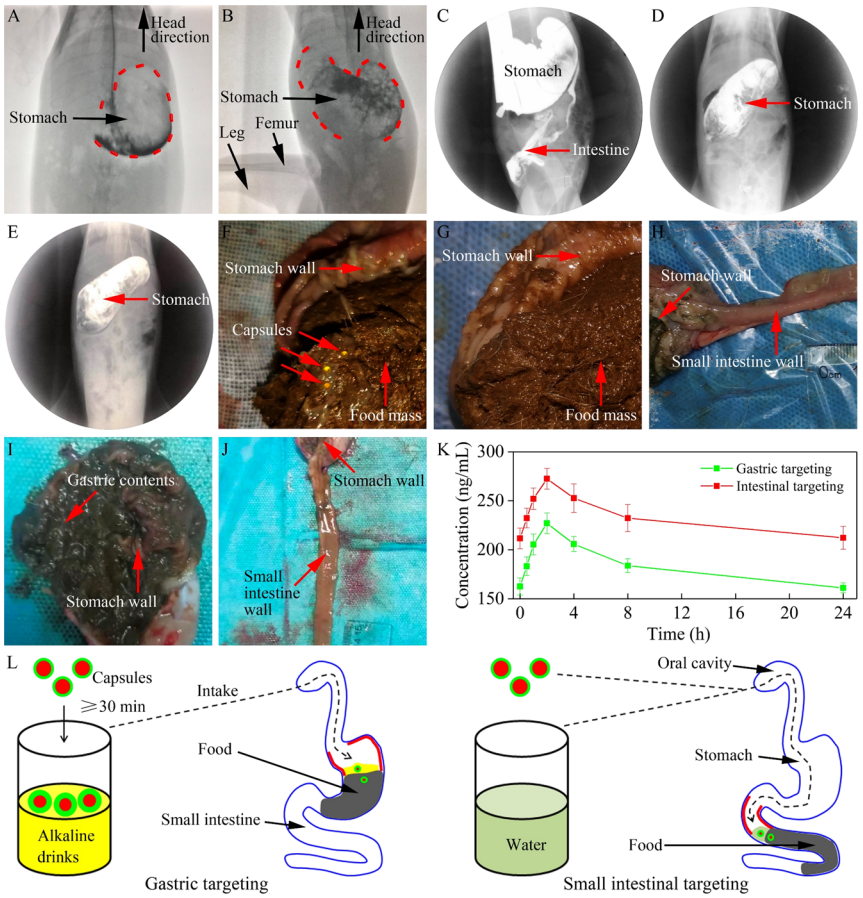


Figure 5. In vivo digestion of fish oil/β-carotene-loaded core−shell millimeter-sized soft capsules in rabbit models with an empty and full stomach. (A, B) Top and side views of the rabbit model with an empty stomach under the Sonialvision G4/Sonialvision C200 system. (C) Top view of the rabbit model with an empty stomach under the VS20 digital gastrointestinal X-ray machine at 1 min after barium sulfate suspension (white color) perfusion. (D, E) Top view of the rabbit model with an empty stomach under the VS20 digital gastrointestinal X-ray machine at 1 min and 4 h after barium sulfate suspension (white color) perfusion. (F) Fish oil/β-carotene-loaded soft capsules onto the gastric food mass at 48 h after perfusing capsules into the full stomach of the rabbit model. (G, H) Gastric food mass (G) and dissected small intestine (H) at 0.5 h after perfusing swollen soft capsules into the rabbit model with a full stomach. (I, J) Gastric contents (I) and dissected small intestine (J) at 0.5 h after perfusing soft capsules into the rabbit model with an empty stomach. (K) β-carotene amounts in the rabbit blood as a function of time. (L) Schematics of electrosprayed core−shell millimeter-sized soft capsules as containers of fish oil and lipophilic nutrients for potential gastric (diet expanded capsules with alkaline drinks for full stomach) and small intestinal (diet unexpanded capsules for empty stomach) targeting. Red color indicates fish oils and lipophilic nutrients. Alkaline drinks mean drinks with pH 7.5.

for easy processing and storage. The potentials of the resulting millimeter-sized capsules for delivering fish oils and lipophilic nutrients were investigated. According to the stability tests, in vitro digestion studies, and in vivo digestion studies, the millimeter-sized capsules are particularly suitable for not only delivering fish oil/nutrients to small intestines through oral administration but can also be used for gastric delivery if proper pretreatments are applied. Moreover, excellent bioavailability can be obtained for the lipophilic nutrients. Further work is needed to analyze the protection of highly reactive ingredients from oxygen and heat. In addition, further work is also necessary to explore the effect of surfactant addition in the capsule shell on the soft capsules, which might decrease the initial burst release[36] and prevent shape deformation.[37] In brief, the millimeter-sized capsules balance well the capacity for payload and mechanical stability and therefore should exhibit significant and broad application prospects in food and drug industries partly due to the fact that alginate is an FDA-approved food additive.

4. MATERIALS AND METHODS

4.1. Materials. Deep sea fish oils (food grade, DHA + EPA ≥ 70%) were bought from Xi'an LvTeng Biological Technology Co., Ltd., Shaanxi Province, China. Nile Red was bought from Sangon Biotech (Shanghai) Co., Ltd., China. Sodium alginate and other common chemicals (analytical reagent grade) were bought from Sinopharm Chemical Reagent Co., Ltd., Shanghai, China. All the solutions were prepared by using ultrapure water (18.2 MΩ·cm).

4.2. Preparation of Electrosprayed Core−Shell Millimeter-Sized Soft Capsules. Electrosprayed core−shell millimeter-sized soft capsules were fabricated by a custom-designed coaxial electrospraying instrument. Fish oils and sodium alginate solutions were used as core and shell materials, respectively. $CaCl_2$ solution (25 mM) was used as the collecting solution. The 21# (inner diameter of 0.51 mm and outer diameter of 0.82 mm)/15# (inner diameter of 1.38 mm and outer diameter of 1.80 mm), 23# (inner diameter of 0.33 mm and outer diameter of 0.64 mm)/17# (inner diameter of 1.10 mm and outer diameter of 1.48 mm), and 23# (inner diameter of 0.33 mm and outer diameter of 0.64 mm)/15# (inner diameter of 1.38 mm and outer diameter of 1.80 mm) coaxial needles were applied in this work. The working temperature was 25−30 °C. The relative humidity was 40−50%. Typical electrosprayed core−shell millimeter-sized soft capsules were fabricated as below optimized conditions: fish oils and

https://dx.doi.org/10.1021/acsami.9b23623
ACS Appl. Mater. Interfaces XXXX, XXX, XXX−XXX

sodium alginate solution (2%) were loaded in 5 mL of syringes, which were connected to the inner (23#) and outer needle (17#), respectively, of the coaxial needles. An applied voltage of 20 kV was produced using a high voltage power supply (Tianjin Dongwen High Voltage Power Supply Co., Ltd., Tianjin, China). The feeding rates for the inner and the outer phase were 10 and 40 μL/min, respectively. The distance between the coaxial needle and the liquid surface of the collecting solution was about 9 cm. To study the effects of the electrospraying parameters on the preparation of soft capsules, these parameters (applied voltages, sodium alginate concentration, shell/core feeding rate ratio, times of feeding rate, and coaxial needles) were adjusted and analyzed as described in the main text.

4.3. Observation of Electrosprayed Core−Shell Millimeter-Sized Soft Capsules. The capsule shapes were recorded by a digital camera and inverted optical microscope (MS500W, Shanghai Minz Precision Instruments Co., Ltd., Shanghai, China). The thickest shell diameters, thinnest shell diameters, capsule diameter, and core diameters were measured by the MeizsMcs 6.0 software (Shanghai Minz Precision Instruments Co., Ltd., Shanghai, China). If the capsule is in an ellipsoid, then the thinnest diameter was referred as a capsule diameter. The soft capsules were put on microslides, and the solution around the soft capsules was removed by pipettes and absorbent papers. After about 1 h, the capsules were fixed on conductive adhesive and then pretreated by spraying gold on the capsules for 50 s. Finally, the surface morphology of these capsules was observed by a scanning electron microscope (S-3400, Hitachi, Tokyo, Japan) at an accelerated voltage of 12.0 kV. To observe the presence of fish oil in the core−shell soft capsules, 200 μL of fluorescent dye (0.1% Nile Red) 1,2-propanediol solution (2% water) was added into 5 mL of fish oil and then was vortexed for 5−10 s. After that, core−shell millimeter-sized soft capsules were prepared by the custom-designed electrospraying instrument using the optimized experimental conditions. Then, the core−shell capsules were examined by an inverted optical fluorescence microscope (MS600F, Shanghai Minz Precision Instruments Co. Ltd., Shanghai, China). The core−shell capsules were observed by bright-field mode. Nile Red dyes were observed by fluorescence mode with an excitation wavelength of 460−550 nm and an emission wavelength of 590 nm.[38] Then, the bright-field and fluorescence image were merged by the MeizsMcs 6.0 software (Shanghai Minz Precision Instruments Co. Ltd., Shanghai, China).

4.4. Stability Observation of Electrosprayed Core−Shell Millimeter-Sized Soft Capsules. The electrosprayed core−shell millimeter-sized soft capsules were stored in 25 mM $CaCl_2$ solution. At designated time points, the soft capsules were taken out and were put into 35 mm Petri dishes. The solution around the soft capsules was removed by pipettes and absorbent papers. Finally, the soft capsules in Petri dishes were recorded by a digital camera.

4.5. In Vitro Digestion of Electrosprayed Core−Shell Millimeter-Sized Soft Capsules. To explore potential release in the stomach and intestine, simulated stomach (10 mM NaH_2PO_4, pH 2.0, adjusted with phosphoric acid) and simulated small intestine solutions (8.4 mM Na_2HPO_4, 1.6 mM NaH_2PO_4, pH 7.5) were prepared. β-carotene (10 mg) was added into 12 mL of fish oil and was stirred (240 rpm) at room temperature for about 8 h to ensure that all β-carotene compounds were dissolved. After that, core−shell millimeter-sized soft capsules were prepared by the custom-designed electrospraying instrument using the optimized experimental conditions. One hundred soft capsules were put into 10 mL of different solutions in vials and then were incubated at different temperatures (4, 20, and 37 °C). The vials were kept in a dark place. At designated time points, the glass vials with soft capsules were recorded by a digital camera, and the soft capsules were taken out for diameter measurement and bearing test. The diameter measurement was performed by comparing the diameter with the length of the microslide in the digital camera images. If the capsule is in an ellipsoid, then the thinnest diameter was referred as a capsule diameter. The bearing test was performed as below: the taken-out soft capsules were put on a microslide. The solution around the capsules was removed by pipettes and absorbent papers. Then, one bearing microslide (5.03 ± 0.08 g, n = 5) was gently put on the soft capsules. Undigested soft capsules that were stored in 25 mM $CaCl_2$ solution were used as controls. The bearing tests were recorded by a digital camera, and the images before and after the bearing test were extracted from the videos.

4.6. Rabbit Models with Full or Empty Stomachs and Direct Perfusion Method. In this work, healthy New Zealand white rabbits (from the Animal Center in 960 Hospital of PLA) with an age of 3−4 months and a weight of 1.4−1.6 kg were chosen to construct rabbit models. Millimeter-sized soft capsules with a β-carotene/fish oil mass/volume ratio of 1.25 mg/mL were applied for animal studies. A total of nine rabbits were randomly classified into three groups. Groups 1 and 2 were subjected to jejunitas for 7 days. A disposable transfusion set (Shandong Weigao Group Medical Polymer Co., Ltd., China) with an inner diameter of 3.5 mm was used to cut its head and then was transorally inserted into the gastric cavity using a 0.035 in. flexible-tip guidewire (RF ∗ GA35153M, Terumo, Tokyo, Japan). Then, 10 mL of iopromide injection solution (300 mg/mL) was directly perfused into the stomachs of the jejunitas rabbits in group 1. These rabbits were observed by the Sonialvision G4/Sonialvision C200 system (Shimadzu, Japan) with different body positions. Fifty milliliters of 30% barium sulfate suspension (Shandong Changqing Pharmaceutical Co., Ltd., China) with 20 mm-sized soft capsules was directly perfused into the stomachs of the jejunitas rabbits in group 2. These rabbits were observed by the VS20 digital gastrointestinal X-ray machine (Shimadzu, Japan). Twenty milliliters of 30% barium sulfate suspension with 20 mm-sized soft capsules was directly perfused into the stomachs of the rabbit without jejunitas in group 3. These rabbits were observed by the VS20 digital gastrointestinal X-ray machine (Shimadzu, Japan).

4.7. Stomach-Specific Delivery to the Rabbit Model with a Full Stomach. A total of nine rabbits were randomly classified into three groups. Ten millimeter-sized soft capsules in 45 mL of $CaCl_2$ solution were directly perfused into the stomachs of the rabbits in group 1 after meal intake. Five milliliters of $CaCl_2$ solution was perfused to make sure that all the capsules reached into the stomachs. The rabbits were subjected to jejunitas and were sacrificed at 48 h to observe the stomach and small intestine. Twenty millimeter-sized soft capsules were kept in 45 mL of the simulated small intestine (pH 7.5) for 2 h, and the mixture was directly perfused into the stomachs of the rabbits in groups 2 and 3 after meal intake. Five milliliters of the simulated small intestine (pH 7.5) was perfused to make sure that all the capsules reached into the stomachs, and then, the rabbits were subjected to jejunitas. The rabbits in group 2 were sacrificed at 0.5 h to observe the stomach and small intestine. The rabbits in group 3 were sacrificed at 24 h, and the β-carotene amounts in the rabbit blood were analyzed at the designated time points (0, 0.5, 1, 2, 4, 8, and 24 h).

4.8. Small Intestine-Specific Delivery to the Rabbit Model with an Empty Stomach. A total of six rabbits were subjected to jejunitas for 7 days to construct a rabbit model with an empty stomach and were randomly classified into two groups. Twenty millimeter-sized soft capsules in 50 mL of $CaCl_2$ solution were directly perfused into the stomachs of the rabbits in groups 1 and 2. The rabbits in group 1 were subjected to jejunitas and were sacrificed at 0.5 h to observe the stomach and small intestine. The rabbits in group 2 were sacrificed at 24 h, and the β-carotene amounts in the rabbit blood were analyzed at the designated time points (0, 0.5, 1, 2, 4, 8, and 24 h).

4.9. Determination of β-Carotene Amounts in the Rabbit Blood. The β-carotene amounts in the blood were determined by a rabbit β-carotene ELISA kit (Shanghai Jianglai Biotech Co., Ltd., China). Briefly, 1 mL of blood was collected from the rabbit at each time point. Then, the blood was naturally coagulated for 20 min, centrifugated (TG16-W centrifuge, Hunan Xiangyi Laboratory Development Co., Ltd. China) at 2500 rpm for 20 min, and the supernatant fraction (blood serum, about 40% volume ratio of the blood) was carefully collected. The supernatant fraction was diluted with a dilution ratio of 20%, and the β-carotene amounts in the diluted solutions were analyzed by the rabbit β-carotene ELISA kit. The obtained β-carotene concentrations were divided by a serum/

https://dx.doi.org/10.1021/acsami.9b23623
ACS Appl. Mater. Interfaces XXXX, XXX, XXX−XXX

blood volume ratio of 40% and a dilution ratio of 20% to obtain the β-carotene concentrations in the rabbit blood.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsami.9b23623.

Digital camera and optical microscopy images of typical soft capsules prepared under different applied voltages, digital camera and optical microscopy images of typical soft capsules prepared with 4.0% sodium alginate solution, digital camera and optical microscopy images of typical soft capsules prepared with different shell/core feeding rate ratios, shell/core ratios of the thickest and thinnest shell as a function of the shell/core feeding rate ratio, digital camera and optical microscopy images of typical soft capsules prepared with different coaxial needles, soft capsule and fish oil core diameters vs different coaxial needles, digital camera and optical microscopy images of typical fish oil/β-carotene-loaded soft capsules prepared with different distances between the coaxial needle and the liquid surface of the collecting solution, fish oil/β-carotene-loaded soft capsule diameters vs distances between the coaxial needle and the liquid surface of the collecting solution, soft capsules after different storage times in 25 mM $CaCl_2$ solution, diameters of soft capsules prepared under different voltages after different storage times in 25 mM $CaCl_2$ solution, and soft capsules (samples 1−6) after different storage times in different pH solution at different storage temperatures (PDF)

Bearing test of soft capsules (samples 1−6) after 0.5 h of storage in different pH solution at different storage temperatures (MP4)

Bearing test of soft capsules (samples 1−6) after 2 h of storage in different pH solution at different storage temperatures (MP4)

Bearing test of soft capsules (samples 1−6) after 3 h of storage in different pH solution at different storage temperatures (MP4)

Bearing test of soft capsules (samples 1−6) after 6 h of storage in different pH solution at different storage temperatures (MP4)

Bearing test of soft capsules (samples 1−6) after 18 h of storage in different pH solution at different storage temperatures (MP4)

■ AUTHOR INFORMATION

Corresponding Author

Jian Zhong − *National R&D Branch Center for Freshwater Aquatic Products Processing Technology (Shanghai), Integrated Scientific Research Base on Comprehensive Utilization Technology for By-Products of Aquatic Product Processing, Ministry of Agriculture and Rural Affairs of the People's Republic of China, Shanghai Engineering Research Center of Aquatic-Product Processing and Preservation, College of Food Science & Technology, Shanghai Ocean University, Shanghai 201306, China;* orcid.org/0000-0002-2475-3221; Email: jzhong@shou.edu.cn

Authors

Panpan Wang − *National R&D Branch Center for Freshwater Aquatic Products Processing Technology (Shanghai), Integrated Scientific Research Base on Comprehensive Utilization Technology for By-Products of Aquatic Product Processing, Ministry of Agriculture and Rural Affairs of the People's Republic of China, Shanghai Engineering Research Center of Aquatic-Product Processing and Preservation, College of Food Science & Technology, Shanghai Ocean University, Shanghai 201306, China*

Min Li − *Department of Medical Image, 960 Hospital of PLA (Jinan Military General Hospital), Jinan City 250031, People's Republic of China*

Daixu Wei − *College of Life Sciences and Medicine, Northwest University, Xi'an 710069, People's Republic of China; School of Life Sciences, Tsinghua-Peking Center for Life Sciences, Tsinghua University, Beijing 100084, China*

Mengzhen Ding − *National R&D Branch Center for Freshwater Aquatic Products Processing Technology (Shanghai), Integrated Scientific Research Base on Comprehensive Utilization Technology for By-Products of Aquatic Product Processing, Ministry of Agriculture and Rural Affairs of the People's Republic of China, Shanghai Engineering Research Center of Aquatic-Product Processing and Preservation, College of Food Science & Technology, Shanghai Ocean University, Shanghai 201306, China*

Lina Tao − *National R&D Branch Center for Freshwater Aquatic Products Processing Technology (Shanghai), Integrated Scientific Research Base on Comprehensive Utilization Technology for By-Products of Aquatic Product Processing, Ministry of Agriculture and Rural Affairs of the People's Republic of China, Shanghai Engineering Research Center of Aquatic-Product Processing and Preservation, College of Food Science & Technology, Shanghai Ocean University, Shanghai 201306, China*

Xunwei Liu − *Department of Medical Image, 960 Hospital of PLA (Jinan Military General Hospital), Jinan City 250031, People's Republic of China*

Fengping Zhang − *Sichuan Willtest Technology Co., Ltd., Chengdu, Sichuan Province, China,Key Laboratory of Nutritional and Healty Cultivation of Aquatic-Product and Livestock-Poultry, Ministry of Agriculture and Rural Affairs of the People's Republic of China, Tongwei Co., Ltd., Chengdu 610041, China*

Ningping Tao − *National R&D Branch Center for Freshwater Aquatic Products Processing Technology (Shanghai), Integrated Scientific Research Base on Comprehensive Utilization Technology for By-Products of Aquatic Product Processing, Ministry of Agriculture and Rural Affairs of the People's Republic of China, Shanghai Engineering Research Center of Aquatic-Product Processing and Preservation, College of Food Science & Technology, Shanghai Ocean University, Shanghai 201306, China*

Xichang Wang − *National R&D Branch Center for Freshwater Aquatic Products Processing Technology (Shanghai), Integrated Scientific Research Base on Comprehensive Utilization Technology for By-Products of Aquatic Product Processing, Ministry of Agriculture and Rural Affairs of the People's Republic of China, Shanghai Engineering Research Center of Aquatic-Product Processing and Preservation, College of Food Science & Technology, Shanghai Ocean University, Shanghai 201306, China*

Mingyuan Gao − *CAS Key Laboratory of Colloid, Interface and Chemical Thermodynamics, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, China;* orcid.org/0000-0002-7360-3684

https://dx.doi.org/10.1021/acsami.9b23623
ACS Appl. Mater. Interfaces XXXX, XXX, XXX−XXX

Complete contact information is available at:
https://pubs.acs.org/10.1021/acsami.9b23623

Author Contributions

[▽]P.W. and M.L. contributed equally. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research has been supported by research grants from the National Key R&D Program (2019YFD0902003) and Shanghai Municipal Education Commission—Gaoyuan Discipline of Food Science & Technology Grant Support (Shanghai Ocean University).

■ REFERENCES

(1) Gullapalli, R. P. Soft Gelatin Capsules (Softgels). *J. Pharm. Sci.* **2010**, *99*, 4107−4148.
(2) Freitas, S.; Merkle, H. P.; Gander, B. Microencapsulation by Solvent Extraction/Evaporation: Reviewing the State of the Art of Microsphere Preparation Process Technology. *J. Controlled Release* **2005**, *102*, 313−332.
(3) Saifullah, M.; Shishir, M. R. I.; Ferdowsi, R.; Tanver Rahman, M. R.; Van Vuong, Q. Micro and Nano Encapsulation, Retention and Controlled Release of Flavor and Aroma Compounds: A Critical Review. *Trends Food Sci. Technol.* **2019**, *86*, 230−251.
(4) Nagai, K.; Takaki, T.; Norimatsu, T.; Yamanaka, T. Fabrication of Highly Spherical Millimeter-Sized Poly(Amic Acid) Capsules by Removing Non-Volatile Solvent. *Macromol. Rapid Commun.* **2001**, *22*, 1344−1347.
(5) Bourlinos, A. B.; Petridis, D. Shape Fabrication of Millimeter-Sized Metal-Containing Carboxymethyl Cellulose Hollow Capsules. *Chem. Commun.* **2002**, *38*, 2788−2789.
(6) Takei, T.; Yamasaki, Y.; Yuji, Y.; Sakoguchi, S.; Ohzuno, Y.; Hayase, G.; Yoshida, M. Millimeter-Sized Capsules Prepared Using Liquid Marbles: Encapsulation of Ingredients with High Efficiency and Preparation of Spherical Core-Shell Capsules with Highly Uniform Shell Thickness Using Centrifugal Force. *J. Colloid Interface Sci.* **2019**, *536*, 414−423.
(7) Ding, Q.; Xu, X.; Yue, Y.; Mei, C.; Huang, C.; Jiang, S.; Wu, Q.; Han, J. Nanocellulose-Mediated Electroconductive Self-Healing Hydrogels with High Strength, Plasticity, Viscoelasticity, Stretchability, and Biocompatibility toward Multifunctional Applications. *ACS Appl. Mater. Interfaces* **2018**, *10*, 27987−28002.
(8) Song, Y.; Chan, Y. K.; Ma, Q.; Liu, Z.; Shum, H. C. All-Aqueous Electrosprayed Emulsion for Templated Fabrication of Cytocompatible Microcapsules. *ACS Appl. Mater. Interfaces* **2015**, *7*, 13925−13933.
(9) Zhong, J.; Zhang, H.; Yan, J.; Gong, X. Effect of Nanofiber Orientation of Electrospun Nanofibrous Scaffolds on Cell Growth and Elastin Expression of Muscle Cells. *Colloids Surf., B* **2015**, *136*, 772−778.
(10) Liu, X.; Wei, D.; Zhong, J.; Ma, M.; Zhou, J.; Peng, X.; Ye, Y.; Sun, G.; He, D. Electrospun Nanofibrous P(DLLA−CL) Balloons as Calcium Phosphate Cement Filled Containers for Bone Repair: In Vitro and in Vivo Studies. *ACS Appl. Mater. Interfaces* **2015**, *7*, 18540−18552.
(11) Sun, G.; Wei, D.; Liu, X.; Chen, Y.; Li, M.; He, D.; Zhong, J. Novel Biodegradable Electrospun Nanofibrous P(Dlla-Cl) Balloons for the Treatment of Vertebral Compression Fractures. *Nanomed.: Nanotechnol., Biol. Med.* **2013**, *9*, 829−838.
(12) Qiao, Y.; Shi, C.; Wang, X.; Wang, P.; Zhang, Y.; Wang, D.; Qiao, R.; Wang, X.; Zhong, J. Electrospun Nanobelt-Shaped Polymer Membranes for Fast and High-Sensitivity Detection of Metal Ions. *ACS Appl. Mater. Interfaces* **2019**, *11*, 5401−5413.
(13) Zhang, N.; Wang, X.; Ma, C.; Qiao, Y.; Zhang, H.; Shi, C.; Zhou, X.; Zhang, Y.; Qiao, R.; Wang, X.; Zhong, J. Electrospun Nanofibrous Cellulose Acetate/Curcumin Membranes for Fast Detection of Pb Ions. *J. Nanosci. Nanotechnol.* **2019**, *19*, 670−674.
(14) Zhang, N.; Qiao, R.; Su, J.; Yan, J.; Xie, Z.; Qiao, Y.; Wang, X.; Zhong, J. Recent Advances of Electrospun Nanofibrous Membranes in the Development of Chemosensors for Heavy Metal Detection. *Small* **2017**, *13*, 1604293.
(15) Lv, D.; Wang, R.; Tang, G.; Mou, Z.; Lei, J.; Han, J.; De Smedt, S.; Xiong, R.; Huang, C. Ecofriendly Electrospun Membranes Loaded with Visible-Light-Responding Nanoparticles for Multifunctional Usages: Highly Efficient Air Filtration, Dye Scavenging, and Bactericidal Activity. *ACS Appl. Mater. Interfaces* **2019**, *11*, 12880−12889.
(16) Zhu, M.; Han, J.; Wang, F.; Shao, W.; Xiong, R.; Zhang, Q.; Pan, H.; Yang, Y.; Samal, S. K.; Zhang, F.; Huang, C. Electrospun Nanofibers Membranes for Effective Air Filtration. *Macromol. Mater. Eng.* **2017**, *302*, 1600353.
(17) Lv, D.; Zhu, M.; Jiang, Z.; Jiang, S.; Zhang, Q.; Xiong, R.; Huang, C. Green Electrospun Nanofibers and Their Application in Air Filtration. *Macromol. Mater. Eng.* **2018**, *303*, 1800336.
(18) Jiang, S.; Liu, F.; Lerch, A.; Ionov, L.; Agarwal, S. Unusual and Superfast Temperature-Triggered Actuators. *Adv. Mater.* **2015**, *27*, 4865−4870.
(19) Agarwal, S.; Jiang, S.; Chen, Y. Progress in the Field of Water− and/or Temperature−Triggered Polymer Actuators. *Macromol. Mater. Eng.* **2019**, *304*, 1800548.
(20) Zeng, J.; Xu, X.; Chen, X.; Liang, Q.; Bian, X.; Yang, L.; Jing, X. Biodegradable Electrospun Fibers for Drug Delivery. *J. Controlled Release* **2003**, *92*, 227−231.
(21) Chen, S.; Li, R.; Li, X.; Xie, J. Electrospinning: An Enabling Nanotechnology Platform for Drug Delivery and Regenerative Medicine. *Adv. Drug Deliv. Rev.* **2018**, *132*, 188−213.
(22) Gao, S.; Tang, G.; Hua, D.; Xiong, R.; Han, J.; Jiang, S.; Zhang, Q.; Huang, C. Stimuli-Responsive Bio-Based Polymeric Systems and Their Applications. *J. Mater. Chem. B* **2019**, *7*, 709−729.
(23) Zhu, Y.; Chen, Y.; Xu, G.; Ye, X.; He, D.; Zhong, J. Micropattern of Nano-Hydroxyapatite/Silk Fibroin Composite onto Ti Alloy Surface Via Template-Assisted Electrostatic Spray Deposition. *Mater. Sci. Eng. C* **2012**, *32*, 390−394.
(24) Javadi, A.; Solouk, A.; Haghbin Nazarpak, M.; Bagheri, F. Surface Engineering of Titanium-Based Implants Using Electrospraying and Dip Coating Methods. *Mater. Sci. Eng. C* **2019**, *99*, 620−630.
(25) Pawar, A.; Thakkar, S.; Misra, M. A Bird's Eye View of Nanoparticles Prepared by Electrospraying: Advancements in Drug Delivery Field. *J. Controlled Release* **2018**, *286*, 179−200.
(26) Ma, Y.; Björnmalm, M.; Wise, A. K.; Cortez-Jugo, C.; Revalor, E.; Ju, Y.; Feeney, O. M.; Richardson, R. T.; Hanssen, E.; Shepherd, R. K.; Porter, C. J. H.; Caruso, F. Gel-Mediated Electrospray Assembly of Silica Supraparticles for Sustained Drug Delivery. *ACS Appl. Mater. Interfaces* **2018**, *10*, 31019−31031.
(27) Li, Z.; Behrens, A. M.; Ginat, N.; Tzeng, S. Y.; Lu, X.; Sivan, S.; Langer, R.; Jaklenec, A. Biofilm-Inspired Encapsulation of Probiotics for the Treatment of Complex Infections. *Adv. Mater.* **2018**, *30*, 1803925.
(28) Hwang, Y. K.; Jeong, U.; Cho, E. C. Production of Uniform-Sized Polymer Core−Shell Microcapsules by Coaxial Electrospraying. *Langmuir* **2008**, *24*, 2446−2451.
(29) Tapia-Hernández, J. A.; Rodríguez-Félix, F.; Katouzian, I. 9 - Nanocapsule Formation By electrospraying. In *Nanoencapsulation Technologies for the Food and Nutraceutical Industries*; Jafari, S. M., Ed.; Academic Press, 2017, pp 320−345.
(30) Lee, K. Y.; Mooney, D. J. Alginate: Properties and Biomedical Applications. *Prog. Polym. Sci.* **2012**, *37*, 106−126.
(31) Eilat-Adar, S.; Lipovetzky, N.; Goldbourt, U.; Henkin, Y. Omega-3 Fatty Acids, Fish, Fish Oil and Cardiovascular Disease-a Review with Implications to Israeli Nutritional Guidelines. *Harefuah* **2004**, *143*, 585−591.

(32) Wang, X.; Zhu, C.; Peng, T.; Zhang, W.; Zhang, J.; Liu, H.; Wu, C.; Pan, X.; Wu, C. Enhanced Stability of an Emulsion Enriched in Unsaturated Fatty Acids by Dual Natural Antioxidants Fortified in Both the Aqueous and Oil Phases. *Food Hydrocolloids* **2018**, *82*, 322−328.
(33) Kuo, C. K.; Ma, P. X. Ionically Crosslinked Alginate Hydrogels as Scaffolds for Tissue Engineering: Part 1. Structure, Gelation Rate and Mechanical Properties. *Biomaterials* **2001**, *22*, 511−521.
(34) Chavoshpour-Natanzi, Z.; Sahihi, M. Encapsulation of Quercetin-Loaded B-Lactoglobulin for Drug Delivery Using Modified Anti-Solvent Method. *Food Hydrocolloids* **2019**, *96*, 493−502.
(35) Sun, X.; Liu, C.; Omer, A. M.; Yang, L.-Y.; Ouyang, X.-k. Dual-Layered Ph-Sensitive Alginate/Chitosan/Kappa-Carrageenan Microbeads for Colon-Targeted Release of 5-Fluorouracil. *Int. J. Biol. Macromol.* **2019**, *132*, 487−494.
(36) Zhai, P.; Chen, X. B.; Schreyer, D. J. Plga/Alginate Composite Microspheres for Hydrophilic Protein Delivery. *Mater. Sci. Eng. C* **2015**, *56*, 251−259.
(37) Davarcï, F.; Turan, D.; Ozcelik, B.; Poncelet, D. The Influence of Solution Viscosities and Surface Tension on Calcium-Alginate Microbead Formation Using Dripping Technique. *Food Hydrocolloids* **2017**, *62*, 119−127.
(38) Martinez, V.; Henary, M. Nile Red and Nile Blue: Applications and Syntheses of Structural Analogues. *Chem. − Eur. J.* **2016**, *22*, 13764−13782.

https://dx.doi.org/10.1021/acsami.9b23623
ACS Appl. Mater. Interfaces XXXX, XXX, XXX−XXX